



Research Article


Heavy metal concentrations in the edible portions of *Centella asiatica*: Health risk toward chronic kidney disease of uncertain etiology



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Abstract

Centella asiatica is a commonly consumed green leafy vegetable in many developing countries due its high nutritious value and availability at low cost. The present study was conducted to assess the Cd, Cr and Pb uptake associated health risks of *Centella asiatica* harvested from organic and non-organic cultivations in a chronic kidney disease of uncertain etiology (CKDue) prevalent area in Sri Lanka. The health risk assessment was conducted in terms of the bioconcentration factor (BCF), soil-to-root and root-to-leaf translocation factors (TF (soil-root) and TF (root-leaf)), Target hazard quotient for each heavy metal (THQ) and hazard index (HI). In addition, the spatial variation of physical and chemical parameters of the root zone soil were assessed using MINITAB 17 statistical software. Results indicated significant spatial variations in conductivity, organic matter content and Cr concentrations among organic and non-organic study sites. The Cr, Cd and Pb concentrations recorded from roots and leaves of *Centella asiatica* were higher than the safe limits for consumption established by the European Union. The health risk analysis indicated that there is a potential of hyper-accumulating Cd in the roots of *Centella asiatica*. Further, the THQ and HI of the heavy metals indicated possible adverse non-cancer health risks associated with long-term consumption of leaves of *Centella asiatica*. Therefore, necessary precautionary actions to prevent the excessive buildup of Cr, Cd and Pb in the edible portions of *Centella asiatica* are essential in order to ensure consumer safety.

Keywords Bioaccumulation · Bioconcentration factor · Hazard index · Heavy metals · Target hazard quotient · Translocation factor

1 Introduction

Chronic kidney disease is a significant health problem in rural agricultural communities in most of the developing countries [1, 2]. Majority of patients do not show any identifiable cause for the disease and therefore, it has been named CKD of unknown etiology (CKDue). In Sri Lanka, occurrence of CKDue was first recognized in the 1990s in the North Central Province of the country and now almost after two decades of first recognition, this disease

has become a major public health issue of high priority in North Central Province with more than 50,000 estimated patients. The prevalence of this disease is now spreading to the adjoining provinces as well [3–7].

The World Health organization have suggested several hypotheses to explain the causative factors for the CKDue prevalent in the North Central Province of Sri Lanka. These hypotheses focus on several key factors including assessing As and Cd concentrations in agrochemicals, drinking

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water and commonly consumed vegetables in the disease prevalent areas [3, 4, 7].

However, research conducted to assess the heavy metal concentration in the major drinking and agricultural water sources the North Central Province of Sri Lanka have revealed that the concentrations of As, Pb, Cd and U in reservoirs and well water were lower than the maximum contaminant levels recommended by the USEPA [8–10]. Therefore, the possibility of drinking water as a source for CKD is debatable and WHO has recommended continuing ongoing water quality surveillance, with a focus on As and Cd in the affected areas.

Regarding the concentration of nephrotoxic heavy metals in the vegetables growing in the area and agrochemicals used in the cultivations, the only “vegetable” analyzed in the WHO study was lotus root [10]. Although the results indicated high Cd content in lotus rhizomes harvested from CKD prevalent areas in the North Central Province [8], no further studies were conducted on the contribution of contaminated vegetables toward the health risk of the community [7, 10–12].

Green leafy vegetables are also a very common dietary component of the Sri Lankan community. However, no research has been conducted to assess the uptake of heavy metals by the commonly consumed green leafy vegetables grown in the CKD prevalent areas in Sri Lanka. Therefore, the present study focused on assessing Pb, Cd and Cr concentrations in the edible portions of a commonly consumed green leafy vegetable species, *Centella asiatica* harvested from a CKD prevalent community in the North Central Province of Sri Lanka.

2 Methodology

2.1 Study area

The study area is located in the North Central Province of Sri Lanka. North Central Province (NCP) is situated in the dry zone and it is the largest province in the country. NCP contains about 15% of the total land area of Sri Lanka and most of the land area is used for agricultural activities. NCP consists of two administrative districts, namely Anuradhapura District and Polonnaruwa District. Among these two administrative districts, the highest prevalence of CKD was reported from the Anuradhapura District and it was recorded that 10% of the adult population in this district was affected from CKD during the past decade [13]. Among the affected population, the highest number of patients were recorded from the Padaviya Medical Officer of Health (MOH), and therefore, the present study was conducted in *Centella asiatica* cultivation sites located in Padaviya.

Four *Centella asiatica* cultivation sites with an area of 100 m² each were selected as study sites from Padaviya area, Sri Lanka. Sites A (80°47'24.354"E and 8°53'10.341"N) and B (80°48'31.169"E and 8°52'13.805"N) were non-organic cultivation sites which applied chemical fertilizer during the culture cycle of *Centella asiatica*. Sites C (80°49'13.999"E and 8°51'27.549"N) and D (80°47'15.788"E and 8°50'31.013"N) were organic cultivation sites that did not apply chemical fertilizer. The distance between any two sampling sites were about 8.5 ± 0.2 km.

2.2 Collection of samples

At the end of each culture cycle during dry (April 2019) and rainy seasons (October 2019), twenty plants and their root zone associated soil samples were collected randomly from each sampling site.

The sampled plants were stored in pre-distilled water rinsed polyethylene bags and transported to the laboratory in ice. In the laboratory, the plants were first washed with tap water and then washed with double distilled water to eliminate adsorbed dust and particulate matters. The plant samples were separated into leaves and roots. The roots and leaves were then cut separately and chopped into small pieces using a plastic knife. These chopped pieces were oven dried at 70–80 °C until they attained a constant weight. The dried samples were ground into powder using acid washed commercial mortar and pestle and then sieved through 0.425 mm mesh size sieve. The sieved samples were stored in desiccators until they were acid digested.

At each of the plant sampled locations, root zone soil samples (at 15 cm depth) were collected and were stored in prewashed polyethylene bags and transported to the laboratory in ice. In the laboratory, each soil sample was oven dried at 105 °C for 24 h and then, ground into a fine powder using acid washed commercial mortar and pestle and sieved through 0.425 mm mesh sieve. The sieved soil samples were stored in the polyethylene bags and placed in desiccators until they were subjected to acid digestion.

2.3 Analysis of heavy metals

The powdered sieved plant and soil samples (0.5 g) were acid digested at 270 °C for 3 h in the Kjeldatherm digestion system using concentrated HNO₃ and HClO₄ in 1:1 (v/v) ratio. The digested solutions were allowed to cool and 5 mL of distilled-deionized water were added to dissolve the precipitate formed on cooling and gently swirled and filtered into a 50 mL volumetric flask through Whatman filter paper no. 42 (125 mm). The clear solution was then diluted up to 50 mL with distilled-deionized water and was stored in acid washed polypropylene bottles until analyzed for heavy metals. The

acid digested samples were analyzed for Cd, Cr and Pb using an Atomic absorption spectrophotometer (Analytic jenaModel novAA 400p) on graphite furnace mode following the procedure described in APHA (1998). The minimum detection limits were 0.02 mg/L for all the analyzed metals. Sandy loam certified reference material CRM 023 (Sigma-Aldrich, USA) were used as standard reference material for soil analysis and white cabbage certified reference material BCR 485 (Sigma-Aldrich, USA) were used as standard reference material for plant part analysis. Continuing control verification was done after every 10 samples to check that variability was within 10% [14].

Standard solutions of respective metals were prepared from the commercial standard calibration solutions (Reagecon Diagnostics limited, Ireland) for each metal separately using deionized water. Analytical grade HNO₃ and deionized water were used as the blank solutions. Duplicate analysis was performed for each set of samples [14].

2.4 Physical and chemical parameters of soil

Soil pH, electrical conductivity, cation exchange capacity and organic matter content of soil collected from each sampling site at each sampling season were analyzed in the laboratory following the procedures described in APHA 1998 [14].

2.5 Data analysis

2.5.1 The bio-concentration factor (BCF) and translocation factor (TF)

Bio-concentration factor (BCF) is the ratio of the metal concentration in various parts of a plant to that in the soil [15]. If the BCF ≤ 1.00, it indicates the plant can only absorb but not accumulate metal. The plant may have potential to accumulate metal if the BCF > 1.00 [15].

BCF was calculated using the following formula as described by Sulaiman et al. 2016 [16]:

$$BCF = \frac{C_{\text{plant}}}{C_{\text{soil}}}$$

where C_{plant} is the concentration of metal in plant part and C_{soil} is the concentration of metal in soil [16].

The translocation capacity from the soil or root to the aboveground part of the plant (leaves), is estimated using the translocation factor (TF) and it was calculated using the following formulas as described by Sulaiman et al. 2016 [16]:

$$TF(R-L) = \frac{C_{\text{leaves}}}{C_{\text{roots}}}$$

where TF ($R-L$) is the translocation factor from roots to leaves, C_{leaves} is the concentration of metal in leaves and C_{root} is the concentration of metal in root. [16]

$$TF(S-R) = \frac{C_{\text{roots}}}{C_{\text{soil}}}$$

where TF ($S-R$) is the translocation factor from soil-to-roots C_{roots} is the concentration of metal in roots and C_{soil} is the concentration of metal in soil [16].

2.5.2 Target hazard quotient (THQ)

The potential health risk for heavy metal consumption through consumption of leaves of *Centella asiatica* was assessed by calculating the target hazard quotient (for adults and children) using the following formula as described by the United States Environmental Protection Agency [17, 18].

$$THQ = \frac{E_f E_D F_{IR} C}{R_{FD} W_{AB} T_A} \times 10^{-3}$$

where E_f is the exposure frequency (156 days/year considering *C. asiatica* is included in the diet 3 days per week); E_D is the exposure duration (77 years, equivalent to the average lifetime of the Sri Lankan population); F_{IR} is the food ingestion rate (US EPA recommended average leafy vegetable consumption rates for adults and children is 2.2 g/person/day, respectively [19]); C is the metal concentration in the edible parts of vegetables (mg/kg(wet weight)); R_{FD} is the oral reference dose (Pb, Cd, Cr and Ni values were 0.0035, 0.001, 1.5 and 0.02 mg/kg/day, respectively [20, 21]); W_{AB} is the average body weight (70 kg for adults and 30 kg for children [22]) and T_A is the average exposure time for non-carcinogens ($E_D \times 365$ days/year). A THQ value greater than 1, indicates that the exposure is likely to cause obvious adverse effects to human health [18].

2.5.3 Hazard index (HI)

The hazard index in the study sites were calculated as the sum of individual THQs for each metal [23].

2.6 Statistical analysis of data

Data were tested for normality using Anderson Darling test and the non-normalized data were log transformed. One-way analysis of variance followed by Tukey's pairwise comparison was used to analyze the variation of physical and chemical parameters of root zone soil and the heavy metal concentrations of roots, leaves and root zone soil collected from the non-organic and organic cultivation sites.

Principal component analysis (PCA) was used to determine the soil quality parameters that characterizes the study sites during dry and rainy seasons. The data were analyzed using MINITAB 17 software. The statistical significance level was defined at $p < 0.05$. All the concentrations are expressed as mg metal /kg dry weight of the soil, root or leaves.

3 Results and discussion

3.1 Physicochemical parameters and heavy metal concentrations of root zone soil of *Centella asiatica*

Variation of physicochemical parameters and heavy metal concentrations in root zone soil of *Centella asiatica* is presented in Table 1. Mean soil pH of the study sites ranged from 6.49 to 7.15. Soil pH did not show significant variations among study sites during both seasons. Mean soil conductivity ranged from 72.20 to 174.60 $\mu\text{S}/\text{cm}$. Mean soil conductivity in the organic study sites were significantly higher than those of the non-organic sites during both seasons (ANOVA, Tukey's pairwise comparison $p < 0.05$, Table 1). However, during the rainy season, there was a significant reduction in soil conductivity in all the sites (Table 1).

Mean percentage organic matter content of soil ranged from 2.66 – 6.94%. The organic matter content of the organic cultivation sites was significantly higher than that in the non-organic sites during both seasons (ANOVA, Tukey's pairwise comparison $p < 0.05$, Table 1). The mean cation exchange capacity of soil ranged from 8.50 to 14.5 meq/100 g. There was no significant variation of cation exchange capacity of soil among the study sites during both seasons (ANOVA, $p > 0.05$, Table 1).

Mean Cd, Cr and Pb concentrations in soil ranged from 0.01 to 0.40, 10.56 to 28.70 and 2.75 to 6.12 mg/kg, respectively (Table 1). Mean Pb concentrations in soil did not show significant variations among study sites during both sampling events (ANOVA, $p > 0.05$, Table 1). However, mean Cr concentration in soil of non-organic sites were significantly higher than that of the organic sites during both sampling events (ANOVA, Tukey's pairwise comparison $p < 0.05$, Table 1). Cd concentration in soil in the non-organic sites were significantly higher than that of the organic sites during the dry season (ANOVA, Tukey's pairwise comparison $p < 0.05$, Table 1). However, during the rainy season, Cd concentration did not show significant variations among study sites (ANOVA, $p > 0.05$, Table 1). The pattern of heavy metal concentrations of soil varied as, $\text{Cr} > \text{Pb} > \text{Cd}$ in both sampling events (Table 1). The Cd, Cr and Pb concentrations in soil during the rainy season were higher than those in the dry season (Table 1). The Cr, Cd and Pb can be added to the natural environment

Table 1 Spatial variation of physicochemical parameters and heavy metal concentrations in the root zone soil sampled from non-organic and organic *Centella asiatica* cultivations in the Padaviya area, Sri Lanka

Sampling event/soil parameters	Non-organic cultivations		Organic cultivations	
	Site A	Site B	Site C	Site D
<i>Dry season</i>				
pH	6.49 ± 0.08 ^a	6.67 ± 0.10 ^a	6.57 ± 0.08 ^a	7.15 ± 0.14 ^a
Conductivity ($\mu\text{S}/\text{cm}$)	124.60 ± 24.40 ^a	125.40 ± 14.20 ^a	175.60 ± 16.40 ^b	173.00 ± 29.70 ^a
Organic matter content (%)	2.66 ± 0.15 ^a	3.58 ± 0.23 ^a	6.91 ± 0.40 ^b	6.94 ± 0.30 ^b
Cation exchange capacity (meq/100 g)	11.50 ± 0.50 ^a	12.00 ± 0.50 ^a	13.50 ± 1.50 ^a	14.50 ± 0.50 ^a
Pb (mg/kg dry weight of soil)	4.04 ± 0.75 ^a	2.75 ± 0.88 ^a	3.48 ± 0.13 ^a	3.34 ± 1.47 ^a
Cr (mg/kg dry weight of soil)	25.16 ± 6.72 ^a	21.24 ± 6.07 ^a	10.56 ± 5.38 ^b	12.42 ± 8.70 ^b
Cd (mg/kg dry weight of soil)	0.24 ± 0.05 ^a	0.29 ± 0.08 ^a	0.29 ± 0.12 ^a	0.26 ± 0.00 ^a
<i>Rainy season</i>				
pH	6.82 ± 0.36 ^a	6.82 ± 0.36 ^a	6.82 ± 0.36 ^a	6.82 ± 0.36 ^a
Conductivity ($\mu\text{S}/\text{cm}$)	72.20 ± 3.73 ^a	76.83 ± 0.23 ^a	141.03 ± 8.60 ^b	136.20 ± 17.40 ^b
Organic matter content (%)	3.17 ± 0.09 ^a	2.63 ± 0.03 ^a	6.18 ± 0.39 ^b	6.60 ± 0.47 ^b
Cation exchange capacity (meq/100 g)	9.50 ± 1.50 ^a	9.50 ± 0.50 ^a	9.50 ± 1.50 ^a	8.50 ± 0.50 ^a
Pb (mg/kg dry weight of soil)	6.12 ± 1.28 ^a	6.74 ± 0.36 ^a	3.36 ± 0.57 ^a	3.02 ± 0.52 ^a
Cr (mg/kg dry weight of soil)	28.78 ± 4.01 ^a	26.10 ± 8.35 ^a	14.29 ± 1.79 ^b	12.70 ± 2.42 ^b
Cd (mg/kg dry weight of soil)	0.40 ± 0.05 ^a	0.35 ± 0.07 ^b	0.21 ± 0.00 ^b	0.23 ± 0.01 ^b

Data are present as mean ± SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$, $N = 20$)

due to various reasons. Mainly, these are components of the agricultural chemicals and long-term application of agricultural chemicals can result in accumulation of these heavy metals in soil [24]. In addition, application of bio-solids such as animal manure, compost can also lead to accumulation of some heavy metals such as Pb in soil [25]. The high concentrations of heavy metals in soil during the rainy season may have been caused by accumulation of non-point source surface runoff. However, the soil Pb, Cd, Cr concentrations in all the study sites were below the levels recommended by European Union (EU) safe limits (300 mg/kg for Pb; 180 mg/kg for Cr and 6.4 mg/kg for Cd) (Table 1).

Principal component analysis showed categorization of study sites according to the soil quality parameters during dry and rainy seasons. Two principal components displaying a cumulative variance of 78.6% were obtained after applying PCA on soil quality parameters. PCA score plot

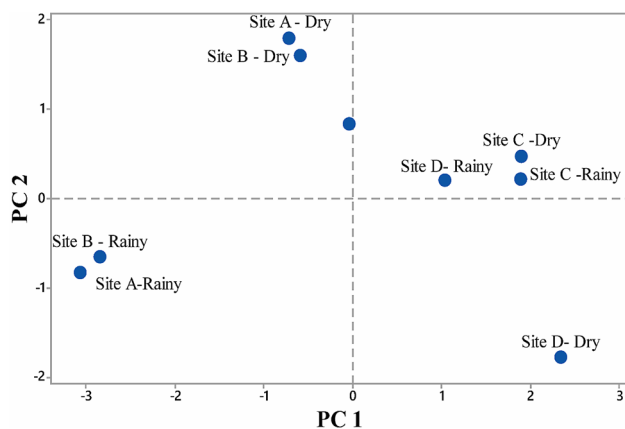


Fig. 1 PCA score plot for variation of soil quality parameters among the study during dry and rainy seasons

for variation of soil quality parameters among the study during dry and rainy seasons is given in Fig. 1. The eigenvalues of the first two principal components, eigenvectors of the soil quality variables and the principal component scores for the study sites are given in Table 2. According to the results of the PCA on soil quality parameters, the non-organic cultivation sites during the dry season (Site A-Dry and Site B-Dry) were grouped together and were characterized by high Cr concentration in soil. Similarly, these two study sites in the rainy season were grouped together and were characterized by high Pb and Cd concentrations in soil. The organic cultivation sites (Site C and Site D) of the rainy season and site C in the dry season were categorized together and were characterized by high conductivity. Site C in the rainy season were separated by other sites and was characterized by high cation exchange capacity in soil (Fig. 1, Table 2).

3.2 Heavy metal concentrations in the roots of *Centella asiatica*

Mean concentrations of Cr, Cd and Pb in the roots of *Centella asiatica* collected from non-organic and organic cultivation sites are given in Table 3. The mean concentration of Cd, Cr and Pb recorded from roots ranged from 1.23 to 2.19, 3.00 to 15.98 and 0.49 to 2.88 mg/kg respectively (Table 3). There was no significant variation of the Cr, Cd and Pb concentrations in roots among the study sites during both sampling seasons. The Pb and Cd concentrations in the roots of *Centella asiatica* collected from all the study sites during the rainy season were significantly higher, than those of the dry season. The Cr concentration in the roots of *Centella asiatica* collected from the non-organic study sites were significantly lower during the rainy season compared to that of the dry season. However,

Table 2 Summary of the PCA of soil quality parameters

PC	Eigenvalues	%Variation	Cumulative %variation
<i>Eigenvalues</i>			
1	4.29	61.4	61.4
2	1.20	17.2	78.6
Variable	PC1	PC2	PC3
<i>Eigenvectors</i> (coefficients in the linear combinations of variables making up PC's)			
pH	0.089	-0.840	-0.159
Conductivity	0.470	0.049	0.208
Organic matter content	0.429	-0.280	-0.171
Cation exchange capacity	0.255	-0.073	0.857
Pb	-0.419	-0.293	0.064
Cr	-0.458	0.117	0.131
Cd	-0.370	-0.329	0.383

Table 3 Mean Cr, Cd and Pb concentrations recorded from roots of *Centella asiatica* sampled from non-organic and organic cultivations

Event/ metal	Mean concentration (mg/kg dry weight)			
	Non-organic cultivations		Organic cultivations	
	Site A	Site B	Site C	Site D
<i>Dry season</i>				
Pb	0.56±0.25 ^a	0.53±0.12 ^a	0.49±0.13 ^a	0.51±0.11 ^{a*}
Cr	14.54±2.55 ^a	15.98±5.82 ^a	3.00±0.71 ^b	3.85±1.36 ^b
Cd	1.30±0.27 ^a	1.62±0.12 ^a	1.29±0.29 ^a	1.23±0.91 ^a
<i>Rainy season</i>				
Pb	2.82±0.39 ^a	2.88±0.78 ^a	2.74±0.98 ^a	2.80±0.11 ^{a**}
Cr	9.05±1.42 ^a	9.04±1.03 ^a	9.11±1.03 ^a	9.05±2.31 ^a
Cd	2.19±0.15 ^a	2.15±0.01 ^a	2.15±0.16 ^a	2.17±0.00 ^a

Results are presented as mean±SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$, $N = 20$)

Cr concentration in the roots of *Centella asiatica* collected from the organic study sites were significantly higher during the rainy season compared to that of the dry season. The mean Pb, Cd, Cr concentrations in roots of *Centella asiatica* in all the study sites were higher than the levels for consumption recommended by European Union (EU) (0.3 mg/kg for Pb; 2.3 mg/kg for Cr and 0.2 mg/kg for Cd) (Table 3).

3.3 Heavy metal concentrations in the leaves of *Centella asiatica*

Mean concentrations of Cr, Cd and Pb in the leaves of *Centella asiatica* collected from non-organic and organic cultivation sites are given in Table 4. The mean concentration of Cd, Cr and Pb recorded from roots ranged from 0.80 to 1.65, 3.09 to 6.17 and 0.83 to 3.93 mg/kg, respectively (Table 4). There was no significant variation of the Cd and Pb concentrations in leaves among the study sites during both sampling seasons. However, the Cr concentration in the leaves of *Centella asiatica* collected from the non-organic study sites were significantly higher than that of the organic study sites in both sampling seasons (Table 4). The mean Pb, Cd, Cr concentrations in leaves of *Centella asiatica* in all the study sites were also higher than the levels recommended by European Union (EU) safe limits for consumption (0.3 mg/kg for Pb; 2.3 mg/kg for Cr and 0.2 mg/kg for Cd) (Table 1).

The Cr, Cd and Pb concentrations in the leaves of *Centella asiatica* were less than those in the roots in all sites at all the sampling events. Similar results were recorded

Table 4 Mean Cr, Cd and Pb concentrations recorded from leaves of *Centella asiatica* collected from non-organic and organic cultivations

Event/metal	Mean concentration (leaves) mg/kg dry weight			
	Non-organic cultivations		Organic cultivations	
	Site A	Site B	Site C	Site D
<i>Dry season</i>				
Pb	3.34±0.33 ^a	3.93±0.31 ^a	3.93±0.74 ^a	3.70±1.25 ^a
Cr	5.73±2.31 ^a	6.00±2.31 ^a	3.09±2.67 ^b	3.49±5.05 ^b
Cd	0.80±0.05 ^a	0.85±0.23 ^a	0.84±0.35 ^a	0.83±0.10 ^a
<i>Rainy season</i>				
Pb	3.79±0.80 ^a	3.71±0.65 ^a	3.74±0.24 ^a	3.61±0.78 ^a
Cr	6.17±0.04 ^a	6.00±1.01 ^a	3.23±0.41 ^b	3.29±1.70 ^b
Cd	1.55±0.29 ^a	1.57±0.32 ^a	1.43±0.12 ^a	1.65±0.09 ^a

Results are presented as mean±SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$, $N = 20$)

by Tang et al. 2009, Ong et al. 2011 and 2015 [26–28]. The roots are the major route of taking up water and nutrients into the plants. Therefore, the roots are in direct contact with the soil solution which contains a mixture of nutrients, microorganisms and heavy metals, organic compounds and their derivatives. In addition, the presence of root hairs increases the absorption and adsorption abilities of roots and further enhance the contact with heavy metal cations in the soil solution. The metal cations that enter into the roots can be immobilized within the root or they can be subjected to temporary storage within roots until they are transported to the aerial parts of the plant. This may result in high concentrations of heavy metal ions in the roots compared to other parts of the plant [27].

3.4 Bioconcentration factor

The bioconcentration factor (BCF) of Cr, Cd and Pb in *Centella asiatica* during rainy and dry seasons is given in Fig. 2. The BCF of Cr, Cd and Pb in *Centella asiatica* during the dry season was higher than those in the rainy season. The BCF of Cd was higher than 1 in all the sites during both seasons. However, the BCF of Cr was lower than 1 in all the sites during both seasons. The BCF of Pb showed slight variations among study sites in both seasons (Fig. 2). BCF is an indicator used in environmental toxicology and risk assessment to determine the degree of intake and storage of toxic substances in animals and plants [29, 30]. A value higher than 1 for BCF indicates that particular species is a hyper-accumulator for heavy metals [29, 31, 32]. Therefore, the results of the present study also indicate

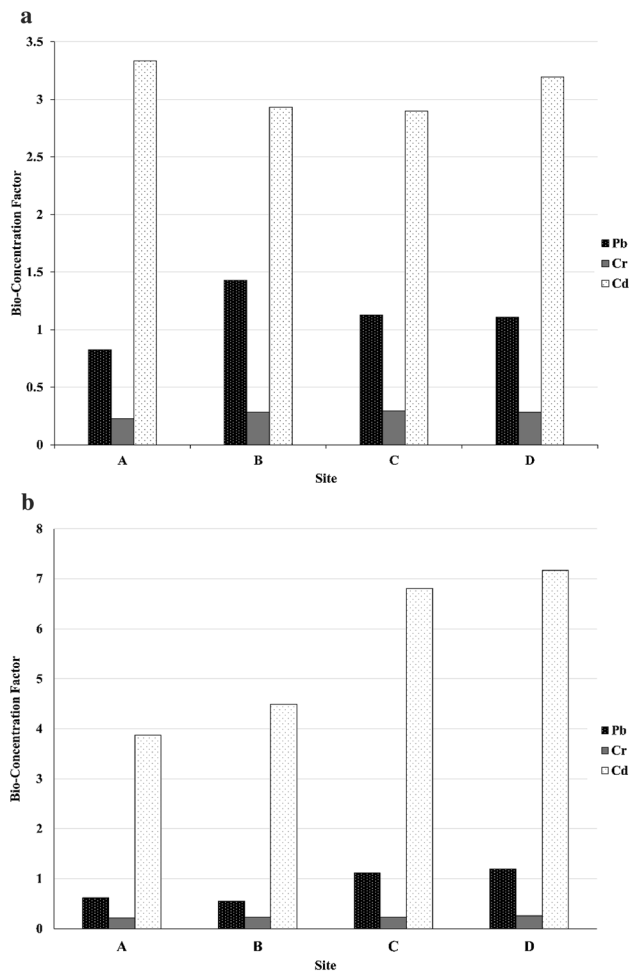


Fig. 2 Bioconcentration factor (BCF) of Cr, Cd and Pb in *Centella asiatica* during rainy and dry seasons (**a**: dry season; **b**: rainy season)

that *Centella asiatica* is a hyper-accumulator species for Cd in both organic and non-organic cultivations.

3.5 Translocation factor

The soil-to-root translocation factor ($TF_{\text{soil-root}}$) of Cr, Cd and Pb in *Centella asiatica* sites during rainy and dry seasons is given in Table 5. In the dry season, the $TF_{\text{soil-root}}$ of Pb and Cd did not show significant variation among non-organic and organic cultivation sites. However, the $TF_{\text{soil-root}}$ of Cr in the dry season was significantly higher in the non-organic cultivation sites compared to that of the organic cultivation sites. Variation of the $TF_{\text{soil-root}}$ of heavy metals showed a different pattern compared to the dry season. In the rainy season, the $TF_{\text{soil-root}}$ of Pb, Cr and Cd were significantly higher in the organic cultivation sites compared to non-organic cultivation sites (Table 5, Student *t* test, $p < 0.05$).

Table 5 Mean translocation factor of Pb, Cr and Cd (from soil to root) of *Centella asiatica* harvested from non-organic and organic cultivation sites during dry and rainy seasons

Event/metal	TF (soil-root)	
	Non-organic cultivations	Organic cultivations
<i>Dry season</i>		
Pb	0.17 ± 0.01^a	0.15 ± 0.01^{aa}
Cr	0.67 ± 0.03^a	0.30 ± 0.01^b
Cd	5.5 ± 0.02^a	4.6 ± 0.02^a
<i>Rainy season</i>		
Pb	0.45 ± 0.02^a	0.88 ± 0.04^b
Cr	0.33 ± 0.02^a	0.68 ± 0.02^b
Cd	5.80 ± 0.01^a	9.84 ± 0.03^b

In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (Student *t* test, $p < 0.05$, $N = 20$)

The root-to-leaf translocation factor ($TF_{\text{root-leaf}}$) of Cr, Cd and Pb in *Centella asiatica* sites during rainy and dry seasons is given in Table 6. The $TF_{\text{root-leaf}}$ of Cd and Pb did not show significant variations among the non-organic and organic cultivation sites during rainy and dry seasons. However, the $TF_{\text{root-leaf}}$ of Cr in the organic cultivations were significantly higher than that of the non-organic sites during the dry season and was significantly lower in the rainy season (Table 6, Student *t* test, $p < 0.05$).

The TF is another factor that can be used to measure the hyper-accumulator potential of plants. Further, TF is used to comprehend the transport mechanisms of heavy metals in different plant components such as roots, stems and leaves [33, 34]. Similar to the BCF, the $TF > 1$ indicates that the species is a hyper-accumulator for the corresponding

Table 6 Mean translocation factor of Pb, Cr and Cd (from root to leaf) of *Centella asiatica* harvested from non-organic and organic cultivation sites during dry and rainy seasons

Event/metal	TF (root-leaf)	
	Non-organic cultivations	Organic cultivations
<i>Dry season</i>		
Pb	6.69 ± 0.01^a	7.64 ± 0.05^a
Cr	0.38 ± 0.01^a	0.97 ± 0.01^b
Cd	0.57 ± 0.03^a	0.66 ± 0.05^a
<i>Rainy season</i>		
Pb	0.32 ± 0.01^a	0.33 ± 0.02^a
Cr	0.67 ± 0.04^a	0.36 ± 0.02^b
Cd	0.72 ± 0.01^a	0.71 ± 0.03^a

In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (Student *t* test, $p < 0.05$, $N = 20$)

Table 7 Target hazard quotient (THQ) and hazard index of Cd, Cr and Pb in adult and child populations

Population	Non-organic cultivations				Organic cultivations			
	THQ - Cd	THQ - Cr	THQ - Pb	HI	THQ - Cd	THQ - Cr	THQ - Pb	HI
Adults	1.016	5.4×10^{-5}	0.014	1.03	1.016	2.9×10^{-5}	0.014	1.03
Children	1.032	0.0001	0.028	1.06	1.032	5.8×10^{-5}	0.028	1.06

heavy metal [35]. In the present study, $TF_{\text{soil-root}}$ for Cd was higher than 1 in both organic and non-organic cultivations during both seasons indicating hyper-accumulation of Cd in the roots of *Centella asiatica*. However, the $TF_{\text{root-leaf}}$ of Cd were less than 1 indicating decreased translocation of Cd from roots to the leaves of *Centella asiatica*. Both $TF_{\text{soil-root}}$ and $TF_{\text{root-leaf}}$ of Cr and Pb were less than 1 indicating that these metals are not hyper-accumulated in *Centella asiatica*.

3.6 Target hazard quotient (THQ) and hazard index (HI)

The target hazard quotient and hazard index of the heavy metals for adults and children are given in Table 7. The values of THQ of Cr and Pb were less than 1 for both adult and child populations and these values were similar for both non-organic and organic cultivations. A THQ less than one indicates that there is no or negligible health hazard associated with long time consumption of *Centella asiatica* [17]. However, the THQ for Cd was slightly higher than 1 for both populations in organic and non-organic cultivations indicating possibility of causing significant health effects due to long-term consumption of *Centella asiatica* (Table 7). HI derived using target hazard quotients in both organic and non-organic cultivations was higher than 1.0 for both adult and child populations, and it indicates that there is a possibility of causing adverse non-cancer health effects over a lifetime of exposure [17, 23].

4 Conclusion

The cultivation type had no significant effect on heavy metal uptake or translocation within *Centella asiatica*. The mean Pb, Cd, Cr concentrations in the leaves and roots of *Centella asiatica* in all the study sites were higher than the safe concentrations for consumption recommended by European Union. The bioconcentration factor indicated that *Centella asiatica* is a hyper-accumulator of Cd. However, the soil-to-root translocation factor and root-to-leaf translocation factor indicated that there is a less potential of translocating Cd from roots to the leafy parts of *Centella asiatica*. Further, the target hazard quotient and hazard index indicated that there is a possibility of causing adverse non-cancer health risks associated with long-term

consumption of leaves of *Centella asiatica*. However, as the roots of *Centella asiatica* has a potential to hyper-accumulate Cd, the consumers need to be advised to properly remove the roots from the plant before consumption. Further, it is advisable to regulate the use of *Centella asiatica* roots in ayurvedic medicines in order to prevent unforeseen ill health conditions in patients due to Cd.

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Data availability Data are available on request from the authors.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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